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Chemical Characterization of Rhizomes of *Atractylodes lancea* and *A. chinensis* Identified by ITS Sequences of nrDNA

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A comparison was made with regard to interspecific and geographical characteristics of nine pharmacologically active components in dried rhizomes of *Atractylodes lancea*, *A. chinensis*, and the hybrids between them, which were identified by sequence data of internal transcribed spacer nuclear ribosomal DNA. The chemical constituents of the nine components were determined. Principal component analysis using the contents data of these nine constituents revealed that *A. lancea* differs chemically from *A. chinensis*. The hybrids possessed chemical variations within the limits observed in both the species. The relationships between these chemical variations and geographical localities were numerically analyzed by the Mahalanobis–Taguchi method, and this analysis enabled the classification of individuals from the localities into four groups.

Key words: *Atractylodes chinensis*, *Atractylodes lancea*, hybrid, local variation, polyacetylenes.

Atractylodes lancea (Thunb.) DC. and *A. chinensis* Koidz. (Compositae) are perennial herbs that are widely distributed in China (Institute of Botany, Academia Sinica ed. 1975). Dried rhizomes of both the species, which have been used as diuretics and analgesics as well as for treating stomach disorders in traditional Kampo medicines, are described in the Japanese Pharmacopeia (The Society of Japanese Pharmacopeia 2001) as the botanical origins of "Sojutsu." These plants had been identified based on the morphological characters of their aerial parts (Kawanishi et al. 1994), Shiba et al. (2006) have reported that *A. lancea* and *A. chinensis* could be more clearly identified by sequences of internal transcribed spacer (ITS) nuclear ribosomal DNA (nrDNA), in addition, hybrid individuals between the two species have been detected.

The chemical constituents of the dried rhizomes of *A. lancea* and *A. chinensis* have been determined by isolation studies or analytical methods, showing the presence of sesquiterpenes (Yosioka et al. 1959), polyacetylenes (Nishikawa et al. 1976, Lehner et al. 1997), and sugar esters (Yahara et al. 1989, Kitajima et al. 2003). Among these components, elemol (**1**) (Matsunaga et al. 2000), hinesol (**2**) (Satoh et al. 2000), β-eudesmol (**3**) (Tsuneki et al. 2005), atractylon (**4**) (Resch et al. 1998), atractylodin (**5**) (Resch et al. 2001), atractylodinol (**6**) (Takeda et al. 2001), and acetylatractylodinol (**7**) (Nakai et al. 2003) have been reported to possess pharmacological activities. Furthermore, we have reported that the constituents of the dried rhizomes of *A.*

chinensis contain a new polyacetylene atractyloyne (**9**) and its structural isomer, 3-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-1,14-diol (**8**), these are easily derivatized to a pharmacologically active substance, 4,6,12-tetradecatriene-8,10-diyne-1,3,14-triol (**10**) (Nakai et al. 2005b).

Although many studies have been carried out on the above-mentioned pharmacologically active compounds, only few studies have reported on the botanical species containing the active principles and their hybrids. Takeda et al. (1996b) reported that *A. lancea*, *A. chinensis*, and *A. koreana*, which grow in China, have large intrapopulational and geographical variations with regard to the essential oils that chiefly consist of **2** and **3**. However, the identification of these species at the time of reporting was not supported by ITS sequence data but by morphological data. In addition, the pharmacologically active polyacetylenes such as **6**, **7**, and **10**, that were derivatized from **8** and **9,** have received little attention.

In the present study, we used crude drugs prepared from the dried rhizomes of *A. lancea*, *A. chinensis*, and their hybrids, which were identified based on ITS sequence data (Shiba et al. 2006) and their contents of pharmacologically active four sesquiterpenes **1**–**4** described previously by Takeda et al. (1994, 1995a, 1995b, 1996a, 1996b), and investigated five pharmacologically active polyacetylenes **5**–**9**. Furthermore, we rearranged the results of sesquiterpenes based on ITS sequence data and summarized the chemical characterization of the species and the localities in which these plants containing these nine compounds grow.

Materials and Methods

Crude drug materials

The crude drugs were used for performing qualitative assays of five polyacetylenes; these drugs were prepared from the same rhizomes, namely, those collected from Jiangsu, Anhui, Hubei, Hebei, Shandong, Henan, and Shaanxi provinces of China from 1990 to 1994 and the contents of the four sesquiterpenes determined previously (Takeda et al. 1994, 1995a, 1995b, 1996a). The rhizomes were numbered according to the aerial parts of the plant, which were identified by Ms. Shiba, on the basis of ITS sequence data. The localities, date of collection, and the number of test materials used in the present study are listed in Table 1. The voucher materials are stored in the herbarium of the Tsumura Central Research Laboratories, Tsumura & Co., Japan.

Standard compounds

Five polyacetylenes, namely, **5**, **6**, **7**, **8**, and **9** which were used for HPLC as the standard compounds, were isolated from the commercially available dried rhizomes of *A. lancea* or *A. chinensis*, as described in the previous reports (Nakai et al. 2003, 2005).

Sample preparations

The powdered rhizome (100 mg) was accurately weighed, extracted with methanol (1.5 mL) for 10 min under sonication, and then centrifuged (3000 rpm, 10 min). The residue was similarly extracted with methanol (1.5 mL) for 10 min and centrifuged twice. Three supernatants were combined, and the solution was made up to a volume of exactly 5 mL by the addition of methanol. A 20-μL aliquot of the filtrate that was passed through a 0.45-μm filter was then injected into the HPLC system.

HPLC analysis

HPLC analysis with LC-10AD pumps, an SPD-M10AVP photodiode-array detector, and a CTO-10A column oven (Shimadzu, Kyoto, Japan) was performed using an ODS column (TSK-gel ODS-80Ts, 4.6 mm i.d. × 250 mm, Tosoh, Tokyo, Japan). The flow rate and the column temperature were 1.0 ml/min and 40°C, respectively. The UV

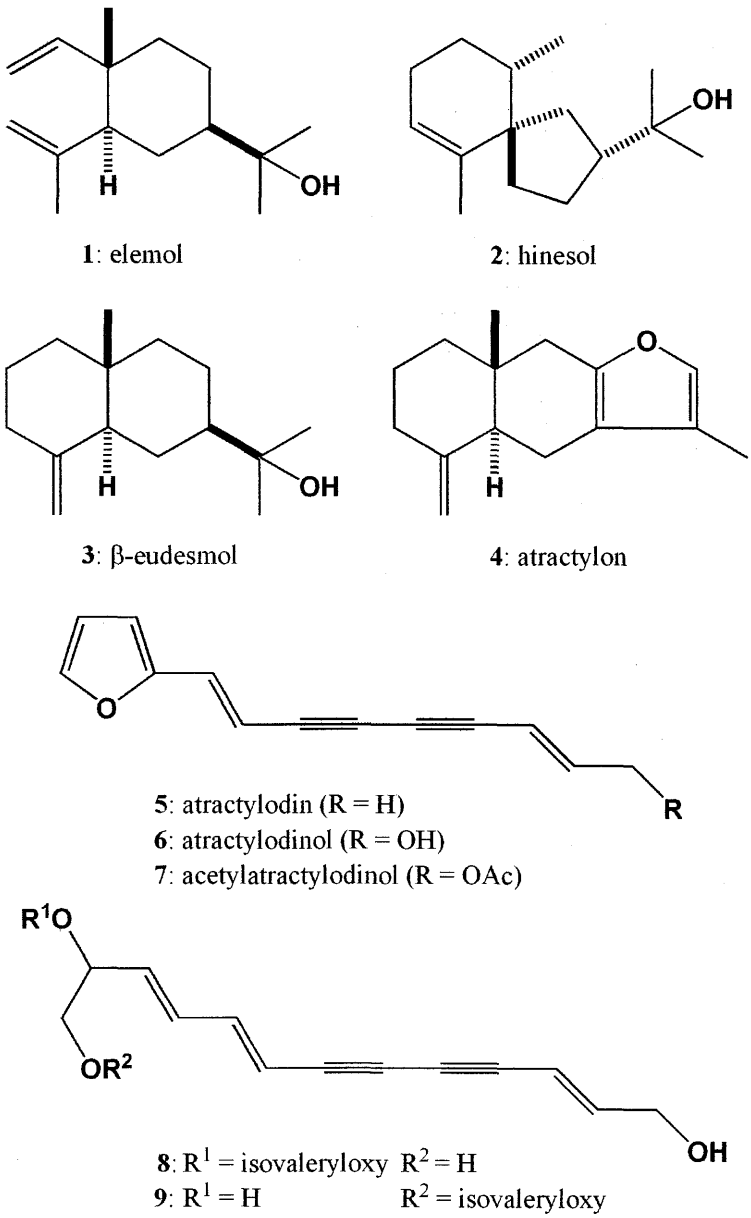

Fig. 1. Structures of compounds: **1**, elemol; **2**, hinesol; **3**, β-eudesmol; **4**, atractylon; **5**, atractylodin; **6**, atractylodinol; **7**, acetylatractylodinol; **8**, 3-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-1,14-diol; **9**, atractyloyne.

data–recorded from 200 to 400 nm–of the effluent from the column were collected, and the peak analysis and assignment were performed using the system analysis software CLASS-LC10 (Shimadzu, Kyoto, Japan). The solvents employed were 20 mM phosphoric acid (A) and acetonitrile (B), and the analytical conditions were as follows: a linear gradient of 50 % A and 50 % B that changed over a period of 60 min to 0 % A

Table 1. Localities, date of collection, the number of test materials, and identification results based on ITS sequences data of *Atractylodes lancea*, *A. chinensis* and hybrids

Localities	Abbreviation	Date of collection	Total number of plants	*A. lancea*	*A. chinensis*	Hybrids
Xuebu, Jintanxian, Jiangsu prov.	Xu	Oct. 15, 1991	16	11	–	5
Liyan, Jiangsu prov.	Li	Oct. 27, 1990	12	7	–	5
Lianyungang, Jiangsu prov.	Yu	Oct. 18, 1990	6	–	–	6
Huangshan, Taiping, Anhui prov.	Hu	Oct. 13, 1991	23	20	–	3
Yingshan, Hubei prov.	Ta	Oct. 4, 1991	4	4	–	–
Suizou, Hubei prov.	Ca	Oct. 1, 1991	6	6	–	–
Danjiangkou, Hubei prov.	Wu	Sep. 29, 1991	15	4	–	11
Ludiang, Hubei prov.	Lu	Sep. 28, 1991	27	3	2	22
Zanhuang, Hebei prov.	Za	Sep. 1, 1992	12	–	12	–
Chengde, Hebei prov.	Da	Sep. 11, 1992	37	–	35	2
Chongli, Hebei prov.	Ch	Sep. 8, 1992	14	–	14	–
Taian, Shandong prov.	Tt	Sep. 18, 1992	17	–	16	1
Qingdao, Shandong prov.	La	Sep. 16, 1992	25	20	–	5
Songshan, Henan prov.	So	Sep. 23, 1994	15	–	14	1
Lushixian, Henan prov.	Ba	Sep. 13, 1994	42	–	21	21
Linshi, Henan prov.	Sh	Sep. 20, 1994	38	–	38	–
Huayin, Shaanxi prov	Hs	Sep. 17, 1994	41	–	41	–
Changan, Shaanxi prov.	An	Sep. 15, 1994	7	–	1	6
			357	75	194	88

–, not estimated.

and 100 % B. The amount of each compound in the methanol extract was calculated based on the peak area at 314 nm for **8** and **9**, at 336 nm for **5** and **6**, and at 370 nm for **7**.

Statistical analysis

The principal component analysis (PCA) and the test of significance were carried out using Microsoft Excel with an add-in program for statistical analysis (*Excel statistics 2002*) provided by the Social Survey Research Information Co. Ltd., Tokyo, Japan. In addition, numerical analysis by the Mahalanobis-Taguchi (MT) method was performed using Microsoft Excel with an add-in program (*MTS for Windows*) provided by Ohken Co., Tokyo, Japan. The methodology for numerical analysis using the MT method has been described in our previous reports (Nakai et al. 2005a).

Results

Interspecies comparison of sesquiterpenes contents

Table 2 shows the mean contents and detectable frequencies of the four sesquiterpenes in the dried rhizomes of *A. lancea*, *A. chinensis*, and their hybrids, which were identified by ITS sequence data. The data presented in Table 2 represent the outcome of rearranging the data that had been reported previously, based on ITS sequence data. First, *A. lancea* was compared with *A. chinensis*. The mean contents of **1** and **4** were significantly higher in *A. chinensis* than in *A. lancea*, whereas that of **2** was significantly lower in *A. chinensis* (each $P < 0.01$). However, the detectable frequencies of **1**–**4** were not accepted as a distinct difference in the interspecies comparison. The hybrids were then compared with *A. lancea* and *A. chinensis*. The mean contents and the frequencies of the hybrids showed only minor differences from both species. On analyzing

Table 2. Mean contents (%) and detectable frequencies (%) of sesquiterpenes obtained from the dried rhizomes of *Atractylodes* species

Components	1	2	3	4
A. lancea	0.077 ± 0.110	1.574 ± 1.970	1.396 ± 1.544	0.343 ± 0.431
n = 75	(37)	(63)	(72)	(49)
A. chinensis	0.172 ± 0.170*	0.437 ± 0.695*	1.148 ± 0.898	0.126 ± 0.245*
n = 194	(64)	(62)	(96)	(32)
hybrid	0.138 ± 0.119	1.582 ± 1.397	1.682 ± 1.337	0.181 ± 0.335*
n = 89	(65)	(79)	(88)	(29)

Values are represented as mean ± S.D. of 357 rhizomes and the detectable frequencies of the 357 rhizomes are provided in parentheses. *P < 0.01 compared with *A. lancea* (Dunnet test).

the four sesquiterpenes, although it was observed that significant difference was acceptable with regard to the components **1**, **2**, and **4** in *A. lancea* and *A. chinensis*, the hybrids showed only slight differences from both species.

Interspecies comparison of polyacetylene contents

Table 3 shows the mean contents and detectable frequencies of the five polyacetylenes in methanol extracts of the dried rhizomes of *A. lancea*, *A. chinensis*, and their hybrids, which were identified by ITS sequence data. First, *A. lancea* was compared with *A. chinensis*. With regard to the mean contents of **5**–**7** in *A. lancea* and *A. chinensis*, the range of variation in the interspecies comparison was small, whereas the contents of **8** and **9** were significantly higher in *A. chinensis* than in *A. lancea* ($P < 0.01$). The detectable frequencies of **5**–**7** were greater than 80 % in both the species, whereas the detectable frequencies of both **8** and **9** showed a wide range of variation in the interspecies comparison. Besides, the constant correlation between **8** and **9** was observed as follows: **9** could be detected without reference to the species whenever **8** could be detected. The detectable frequencies of compounds **8** and **9** in *A. lancea* were approximately 9 % and were considerably lower than those in *A. chinensis* (88 % and 95 %, respectively). In *A. lanca*, where **8** and **9** could be detected, the mean contents of these compounds were 0.021 % and 0.037 %, respectively, and these values were similar to those of *A. chinensis*. Since the detectable frequencies of **8** and **9** were low, the mean contents of these constituents were also considered to be low. Typical HPLC profiles of *A. lancea* and *A. chinensis* are shown in Fig. 2. Next, the hybrids were compared with *A. lancea* and *A. chinensis*. Although the mean contents of the constituents **5**–**7** in the hybrids differed slightly from those of *A. lancea* and *A. chinensis*, the detectable frequencies of **8** and **9** were not similar to those of *A. lancea* and *A. chinensis* and showed a value of approximately 70 %. Along with compound **8**, compound **9** could also be detected in all the hybrids. In the hybrids, the mean contents of these constituents were 0.026 % and 0.044 %, respectively, and these values were similar to those of the two species. The hybrids were specifically separated into two groups with regard to the detectable frequencies of **8** and **9**. The analysis of the five polyacetylenes showed that the detectable frequencies of **8** and **9** differed in the two species and their hybrids.

Chemical characterization of the two species and their hybrids

To comprehensively characterize the two species and their hybrids with regard to their

Table 3. Mean contents (%) and the detectable frequencies (%) of polyacetylenes in the dried rhizomes of *Atractylodes* species

Components	5	6	7	8	9
A. lancea	0.137 ± 0.108	0.046 ± 0.056	0.034 ± 0.045	0.002 ± 0.010	0.006 ± 0.029
n = 75	(100)	(100)	(83)	(9)	(9)
A. chinensis	0.126 ± 0.092	0.062 ± 0.062	0.034 ± 0.046	0.024 ± 0.024*	0.120 ± 0.111*
n = 194	(100)	(100)	(93)	(88)	(95)
hybrid	0.096 ± 0.102	0.081 ± 0.065*	0.032 ± 0.035	0.018 ± 0.022*	0.078 ± 0.084*
n = 89	(100)	(100)	(92)	(70)	

Values are represented as mean ± S.D. of 357 rhizomes and the detectable frequencies of the 357 rhizomes are provided in parentheses. *$P < 0.01$ compared with *A. lancea* (Dunnet test).

constituents, PCA was applied to the contents of the four sesquiterpenes (**1–4**) and the five polyacetylenes (**5–9**) determined by GC and HPLC, respectively. PCA was performed using the data of 357 rhizomes obtained from the two species and their hybrids, and it yielded two new principal components. The individual scores of *A. lancea*, *A. chinensis*, and their hybrids based on the two new variables (PC1 and PC2) were plotted as shown in Fig. 3. Although these scores suggested the division of *A. lancea* individuals into two groups, PCA enabled the chemical differentiation between *A. lancea* and *A. chinensis*. The eigenvectors of each chemical component that affects PC1 and PC2 are shown in Fig. 4. It was suggested that the chemical component **4** was mainly responsible for the characterization of PC1 in the negative direction, whereas the chemical components **2** (positive vector) and both **8** and **9** (negative vectors) were mainly responsible for the characterization of PC2. These results suggest that constituents **2** and **4** are typically involved in the determination of the chemical characteristics of *A. lancea* and enable the classification of *A. lancea* into two groups, while the incidence of compound **8** and **9** enable the chemical differentiation of *A. chinensis*. Additionally, these speculations were well supported by the results presented in Tables 2 and 3. Further, as shown in Fig. 3, the individual scores of the *A. lancea* and *A. chinensis* hybrids have been plotted as small symbols. The hybrids did not possess the intermediate chemical characteristics between *A. lancea* and *A. chinensis*; the variation was observed within the limits of the characteristics that were observed in both the species.

Local variations in the contents of polyacetylenes and sesquiterpenes

Since the results of PCA showed a chemical variation in *A. lancea*, *A. chinensis*, and their hybrids, the relationship between the chemical variations and localities was numerically analyzed. Although various pattern information processing techniques have been used for the numerical analysis, the MT method, which represents the coincidence of the pattern from the standard group (unit space) as the Mahalanobis distance, was used. A unit space (locality in which So is found) was constructed as the standard group based on the regions described in the a ailable Japanese literature. The distances between each rhizome from 17 localities were determined by the inverse matrix method. The chemical components were selected from each locality and classified based on the differences in the contribution ratio of the signal-to-noise ratio and sensitivity of the nine components. As a result, it was observed that the localities could be roughly classified into four groups based on the con-

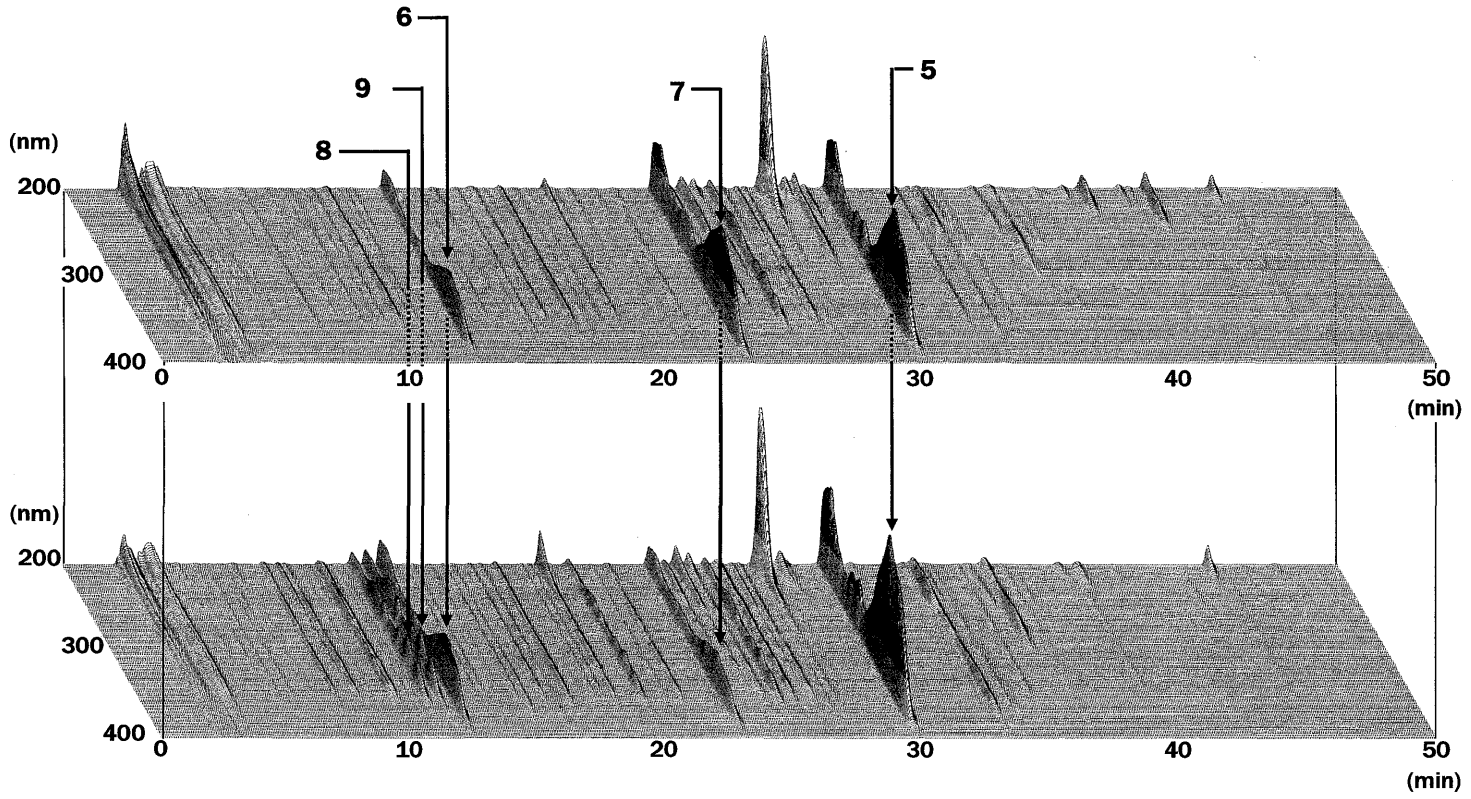

Fig. 2. HPLC profiles of *Atractylodes lancea* (top) and *chinensis* (bottom). **5**, atractylodin; **6**, atractylodinol; **7**, acetylatractylodinol; **8**, 3-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-1,14-diol; **9**, atractyloyne.

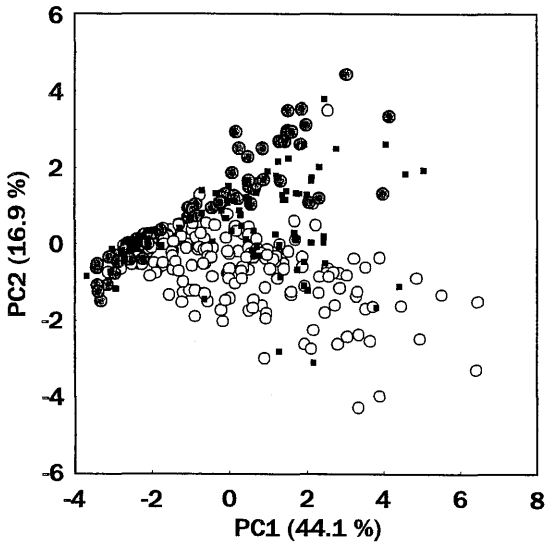

Fig. 3. Chemical characterization of the *Atractyloses lancea*, *A. chinensis*, and their hybrids using PCA results of the content data of the nine components: (●) *A. lancea*; (○) *A. chinensis*; and (■) *A. lancea* and *A. chinensis* hybrids.

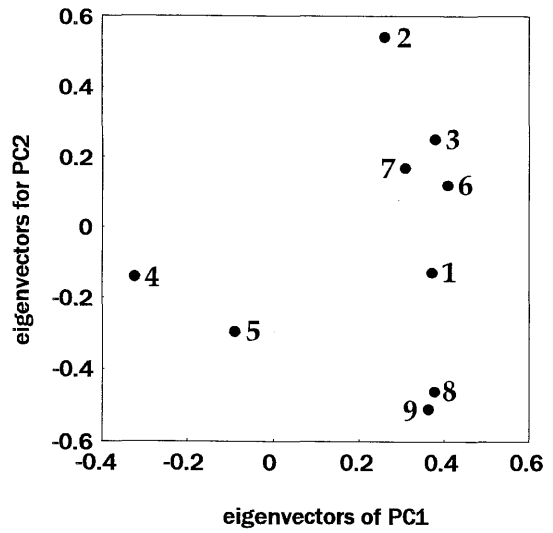

Fig. 4. Eigenvectors for PC1 and PC2 using the PCA results of the content data of the nine components. **1**, elemol; **2**, hinesol; **3**, β-eudesmol; **4**, atractylon; **5**, atractylodin; **6**, atractylodinol; **7**, acetylatractylodinol; **8**, 3-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-1,14-diol; and **9**, atractyloyne.

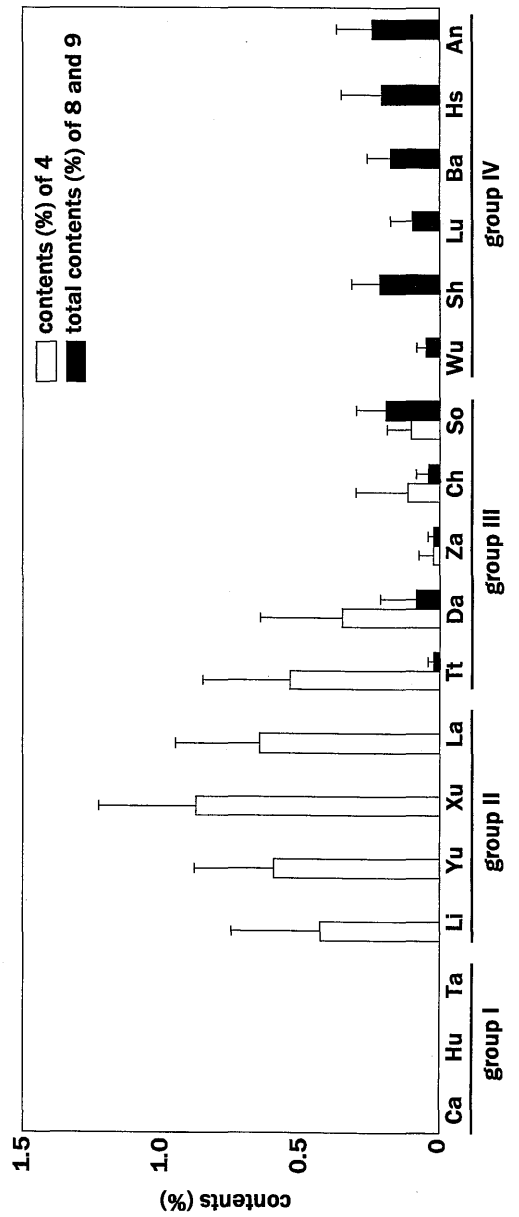

Fig. 5. The contents of **4** (atractylon) and the total contents of **8** and **9** (3-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-1,14-diol and atractyloyne, respectively) in the dried rhizomes of *Atractylodes* plants collected from 18 areas. Each column represents mean ± S.D.

tents of **4** and the total contents of **8** and **9**. The contents of **4** and the total contents of **8** and **9** in each locality in the four groups are shown in Fig. 5. The localities in the four groups, namely, group I (□: Ca, Hu, and Ta), group II (○: Li, Yu, Xu, and La), group III (●: Tt, Da, Za, Ch, and So), and group IV (■: Wu, Sh, Lu, Ba, Hs, and An) are shown in the map (Fig. 6). Both the closed symbols (■ and ●) indicate the localities in which the component **8** or **9** was detected, while both the open symbols (□ and ○) indicate the localities in which the components **8** and **9** were not detected. Similarly, both the square symbols (■ and □) indicate the localities in which component **4** was detected, while the circular symbols (● and ○) indicate the localities in which component **4** was not detected. Thus, it could be concluded that the component contents of the two species and their hybrids possessed the chemical characteristics in every area. Moreover, it was possible to roughly classify the localities into four groups on the basis of the contents of **4** and the total contents of **8** and **9** as an index. Further, the hybrid was detected in all the four groups, and it could be concluded that the chemical characteristics of the hybrids depend on the local variation.

Discussion

In the present study, we used crude drugs prepared from the dried rhizomes of *A. lancea*, *A. chinensis*, and their hybrids, which were identified using ITS sequence data and their contents of four pharmacologically active sesquiterpenes **1**–**4** described previously by Takeda et al. (1994, 1995a,

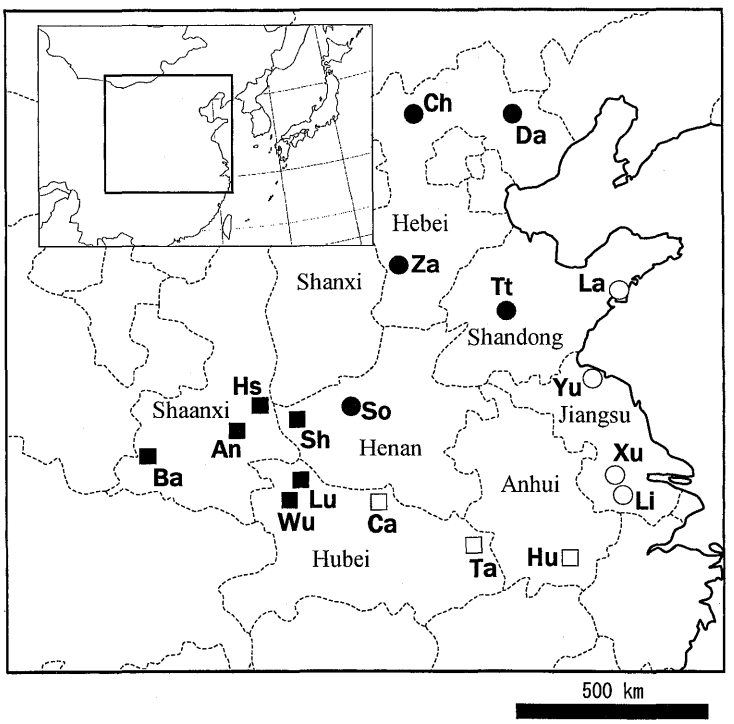

Fig. 6. Local variation in the components of *Atractylodes* plants examined. Symbols and abbreviations indicate the localities and their names: (□) Group I; (○) Group II; (●) Group III; and (■) Group IV.

1995b, 1996a, 1996b), and investigated five pharmacologically active polyacetylenes **5**–**9**. Furthermore, we rearranged the results of sesquiterpenes based on ITS sequence data and summarized the chemical characterization of the species and the localities in which these plants containing these nine components grow.

A comparison of the sesquiterpenes contents in *A. lancea* and *A. chinensis* showed that significant differences were present in their contents of **1**, **2**, and **4**. Although some comparative studies of *A. lancea* and *A. chinensis* with a focus on essential oils have been performed (Nishikawa et al. 1976b, Yasuda et al. 1996, Takeda et al. 1996b, Ji et al. 2004), the results of the analysis of the two species identified using ITS sequence data revealed a significant difference in these three compounds. Although several studies have reported on the detection of **4**–a characteristic component of *A. japonica* and *A. ovata*–in *A. lancea* and *A. chinensis* (Nishikawa et al. 1976b, Takeda et al. 1996b, Ji et al. 2004), we observed that the compound was detected in the two species and their hybrids as distinguished by ITS sequence data. The analysis of polyacetylenes showed that compounds **8** and **9** were considered to be specific and were not detected in a large population of *A. lancea*, but were detected in *A. chinensis* although polyacetylenes **5**–**7** were detected as common costituents in the two species. Therefore, it is reasonable to presume that compounds **8** and **9** may serve as one of the indices for the differentiation of *A. chinensis* from *A. lancea*.

To chemically characterize the two species, PCA was applied to the relative contents of above-mentioned nine compounds. Although these scores suggested the division of *A. lancea* individuals into two groups, PCA enabled chemical differentiation between *A. lancea* and *A. chinensis*. Furthermore, the resultant eigenvectors suggest that compounds **2** and **4** are typically involved in the determination of the chemical characteristics of *A. lancea* and enable the classification of *A. lancea* into two groups, while compounds **8** and **9** enable the chemical differentiation of *A. chinensis*. Additionally, these speculations were well supported by the results of the mean contents and detectable frequencies of each component. Although a comparative study of *A. lancea* and *A. chinensis* has been performed with a specific focus on essential oils, the results of sesquiterpenes along with those of polyacetylenes enabled the recognition of the two species in this study.

Moreover, these chemical characteristics of the *A. lancea* and *A. chinensis* hybrids, which was identified by ITS sequence data, were investigated for the first time. Although the components **1**–**7** detected in the hybrids were common to *A. lancea* and *A. chinensis*, the detectable frequencies of **8** and **9** in the hybrids differed from those observed in the other species. The result of the PCA, in which the contents of the nine components were used as variables, was not helpful in identifying the chemical characteristics of the hybrids. It was recognized that the hybrids did not possess the intermediate chemical characteristics between *A. lancea* and *A. chinensis*, and a variation was accepted within the limits observed in both the species.

In order to investigate the chemical variation in the two species and their hybrids, the relationship between the chemical variations and localities was numerically analyzed. The results of the MT method suggested the classification of the examined localities in China into four groups based on the total contents of **8** and **9** as well as the content of **4**. These results also supported the local grouping based on **4**, which was reported by Takeda et al. (1995b), and facilitated a more detailed classification.

Generally, qualitative and quantitative

controls are required for botanical drugs such as those used in Kampo medicines (The Japan Society for Oriental Medicine 2005). In this study, although the chemical characteristic of two *Atractylodes* species and their hybrids were roughly recognized, chemical variation was found to occur within the species. In particular, the hybrid between *A. lancea* and *A. chinensis* is distributed widely and showed a wide chemical variation; however, within the growing areas, the two species and their hybrids showed lesser chemical variation. Therefore, the study of not only the species but also the growing areas may enable the use of *Atractylodes* species for preparing crude drug materials that consistently contain components possessing pharmacological activities.

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References

Institute of Botany, Academia Sinica, Beijing, ed. 1975. Iconographia Comophytrorum Sinicoeum, vol. 4. pp. 600–602. Science Press, Beijing (in Chinese).

Ji L., Ao P., Pan J. G., Yang J. Y., Yang J. and Hu S. L. 2004. GC-MS analysis of essential oils from rhizomes of *Atractylodes lancea* (Thunb.) DC. and *A. chinensis* (DC.) Koidz. Zhongguo Zhong Yao Za Zhi **26**: 182–185.

Kawanishi F., Takahashi T., Omukai T., Zhang B G., Li Z. L. and Xiao P. G. 1994. Comparison of the outer morphologies, growth and the components in the rhizomes of *Atractylodes* plants cultivated in Kyoto and Beijing. Natural Medicines **48**: 1–10.

Kitajima J., Kamoshita A., Ishikawa T., Takano A., Fukuda T., Isoda S. and Ida Y. 2003. Glycosides of *Atractylodes lancea*. Chem. Pharm. Bull. (Tokyo) **52**: 152–157.

Lehner M S., Steigel A. and Bauer R. 1997. Diacetoxy-substituted polyacetylenes from *Atractylodes lancea*. Phytochemistry **46**: 1023–1028.

Matsunaga T., Hasegawa C., Kawasuji T., Suzuki H., Saito H., Sagioka T., Takahashi R., Tsukamoto H., Morikawa T. and Akiyama T. 2000. Isolation of the antiulcer compound in essential oil from the leaves of *Cryptomeria japonica*. Biol. Pharm. Bull. **23**: 595–598.

Nakai Y., Kido T., Hashimoto K., Kase Y., Sakakibara I., Higuchi M. and Sasaki H. 2003. Effect of the rhizomes of *Atractylodes lancea* and its constituents on the delay of gastric emptying. J. Ethnopharmacol. **84**: 51–55.

——, Yano K., Sakakibara I., Terabayashi S. and Takeda S. 2005a. Grading by applying Mahalanobis distance to chemical constituents of *Atractylodes lancea* rhizome. Quality Engineering **13**: 486–495.

——, Sakakibara I., Hirakura K., Terabayashi S. and Takeda S. 2005b. A new acetylenic compound from the rhizomes of *Atractylodes chinensis* and its absolute configuration. Chem. Pharm. Bull. (Tokyo) **53**: 1580–1581.

Nishikawa Y., Yasuda I., Watanabe Y. and Seto T. 1976a. Studies of the components of *Atractylodes* II. New polyacetylenic compounds in the rhizome of *Atractylodes lancea* De Candolle var. *chinensis* Kitamura. Yakugaku Zasshi **96**: 1322–1326.

——, ——, ——, —— 1976b. Studies on the evaluation of crude drugs (II). Identification of the ingredients of *Atractylodes* by Thin-Layer chromatography, gas chromatography and gas chromatography-mass spectrometry, and the physical and chemical evaluation. Shoyakugaku Zasshi **30**: 132–137.

Resch M., Steigel A., Chen Z. L. and Bauer R. 1998. 5-Lipoxygenase and cyclooxygenase-1 inhibitory active compounds from *Atractylodes lancea*. J. Nat. Prod. **61**: 347–350.

——, Heilmann J., Steigel A. and Bauer R. 2001. Further phenols and polyacetylenes from the rhizomes of *Atractylodes lancea* and their anti-inflammatory activity. Planta Med. **67**: 437–442.

Satoh K., Nagai F. and Kano I. 2000. Inhibition of H^+, K^+-ATPase by hinesol, a major component of So-jutsu, by interaction with enzyme in the E1 state. Biochem. Pharmacol. **59**: 881–886.

Shiba M., Kondo K., Miki E., Yamaji H., Morota T., Terabayashi S., Takeda S., Sasaki H., Miyamoto K. and Aburada M. 2006 (accepted). Identification of medicinal *Atractylodes* based on ITS sequences of nrDNA. Biol. Pharm. Bull. Vol. 29.

Takeda O., Miki E., Morita M., Okada M., Lu Y., He H S. and He S A. 1994. Variation of essential oil components of *Atractylodes lancea* growing in Mt. Maoshan area in Jiangsu province, China. Natural Medicines **48**: 11–17.

——, ——, Terabayashi S., ——, ——, ——, —— 1995a. Variation of essential oil components of *Atractylodes lancea* growing in China. Natural Medicines **49**: 18–23.

——, ——, ——, ——, ——, ——, —— 1995b. Variation of essential oil components of *Atractylodes chinensis* growing in China. Yakugaku Zasshi **115**: 543–552.

——, ——, ——, ——, ——, ——, —— 1996a. Variation of essential oil components of *Atractylodes lancea* (Thunb.) DC. growing in Shanxi and Henan Provinces, China. Natural Medicines **50**: 289–295.

——, ——, ——, ——, ——, ——, —— 1996b. A comparative study on essential oil components of wild and cultivated *Atractylodes lancea* and *A. chinensis*. Planta Med. **62**: 444–449.

——, Tsuchiya K., Kimura K., Kubo M., Okada M. and He S. A. 2001. Arginine vasopressin and angiotensin II receptor binding inhibition by *Atractylodes* species. Pharm. Biol. **39**: 191–197.

The Japan Society for Oriental Medicine 2005. Introduction to Kampo. pp. 98–103. Elsevier Japan, Tokyo.

The Society of Japanese Pharmacopeia 2001. Japanese Pharmacopeia 14th ed. pp. D674–D677. Hirokawa Publishing, Tokyo.

Tsuneki H., Ma E. L., Kobayashi S., Sekizaki N., Maekawa K., Sasaoka T., Wang M. W. and Kimura I. 2005. Antiangiogenic activity of beta-eudesmol in vitro and in vivo. Eur. J. Pharm. **512**: 105–115.

Yahara S., Higashi T., Iwaki K., Nohara T., Marubayashi N., Ueda I., Kohda H., Goto K., Izumi H., Nuno M., Katsuki S., Isoda S. and Satake M. 1989. Studies on the constituents of *Atractylodes lancea*. Chem. Pharm. Bull. (Tokyo) **37**: 2995–3000.

Yasuda N., Oka Y., Otsuki K., Tsuchihashi H., Katagi M. and Nishikawa M. 1996. Study of components in crude drugs by headspace gas chromatography. II. Components of Atractylodes. Yakugaku Zasshi **116**: 728–734.

Yosioka I., Takahashi S., Hikino H. and Sasaki Y. 1959. Studies on the constituents of *Atractylodes*. III. Separation of atractylol into eudesmol and hinesol. Chem. Pharm. Bull. (Tokyo) **7**: 319–323.

中井洋一郎，矢野耕也，司馬真央，近藤健児，武田修己，榊原　巌，寺林　進，竹田秀一，岡田稔：**核リボゾーム DNA，ITS 領域から鑑別されたホソバオケラおよびシナオケラの根茎に含まれる成分の研究**

核リボゾーム DNA，ITS 領域から基原植物を鑑別し，中国18箇所で採取したホソバオケラ，シナオケラおよび両者の雑種を原料として使用し，その根茎に含有される薬効成分の違い，ならびに地理的変異を調査した．9種の成分を指標にホソバオケラおよびシナオケラに含有する成分の違いを検討したところ，主成分分析の解析結果からそれぞれの基原種を特徴付けることが可能であった．また雑種の成分組成は，両基原種の特徴の範囲内でのばらつきが認められた．一方，中国に自生する個体の地理的な成分変異を Mahalanobis–Taguchi 法を使用して検討した結果，今回調査した中国の各地区に自生する個体は，基準とした地区の群の成分パターンの違いから大きく4地域に分類された．

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